The Journal of Microbiology, September 2001, p.195-201
Vol. 39, No. 3

Effect of Moisture Content on Reductive Dechlorination of Polychlorinated Biphenyls and Population Dynamics of Dechlorinating Microorganisms

O-Seob Kwon[1]*, Young Eui Kim[1], and Jong Gyu Park[2]

[1]School of Environmental Science and Engineering and
[2]Institute of Environmental Research, Inje University, Kimhae 621-749, Korea

(Received August 1, 2001 / Accepted August 31, 2001)

The effect of moisture content on the reductive dechlorination of polychlorinated biphenyls and population dynamics of dechlorinating microorganisms was investigated in sediments spiked with Aroclor 1248. In sediment slurry with an overlying water layer, dechlorination ensued after a 4-week lag period and reduced the average number of chlorines per biphenyl from 3.91 to 3.15 after 48 weeks. In the sediments of reduced moisture content, however, dechlorination occurred after a lag period of 12 weeks and decreased the average number of chlorines per biphenyl to only 3.62, and the dechlorination rate was also slower. When the population size of dechlorinators, methanogens, and sulfate-reducing bacteria was determined by the most probable number techniques, however, no difference was found between the slurry and the low-moisture sediments, except for methanogens. The growth of dechlorinating populations coincided with the end of the lag period and they then increased by 3 orders of magnitude in two conditions. Specific growth rate of dechlorinators showed little difference between the slurry and the low-moisture sediments; however, growth yield was high in the sediments of reduced moisture content. The reduction of sediment moisture decreased the dechlorination rate and extent of PCBs but did not inhibit the growth of PCB dechlorinators.

Key words: PCB dechlorination, sediments, moisture content, dechlorinating microorganisms

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants. Improper disposal practices have resulted in the discharge of large quantities of PCBs into soils, river and lake sediments, and landfills. Concerns over their toxicity and bioaccumulation potential have emphasized the need to remediate these contaminated sites. Aquatic sediments which are the main discharging sites of PCBs are complex in their physical and chemical characteristics, and there are many factors affecting the reductive dechlorination of PCBs which include PCB concentration, electron acceptors and donors, carbon sources, bioavailability, temperature, and salinity. The effects of these factors on PCBs dechlorination have been studied (see references 5 and 13).

Microorganisms carrying out a metabolic transformation require adequate moisture for their growth and activity. Especially, the optimum moisture content depends on whether the transformation is aerobic or anaerobic. In soil, excess water is unfavorable for microbial transformation since excess water displaces air from the pores in soil, and waterlogged soil soon becomes anaerobic (3), but higher water contents lead to higher dechlorination rates in soil contaminated with 2,4,6-trichlorophenol and pentachlorophenol (7). It has been reported that water acts as the source of hydrogen for the reductive dechlorination of PCBs (16). However, no detailed studies have been conducted to determine how moisture contents of sediments affect the reductive dechlorination of PCBs.

To remediate contaminated sites, dredging and encapsulation are commonly used, and the most heavily PCB-contaminated sediments have been dredged or will be dredged. Moisture content of dredged sediments decreases over time and diminishes the rates of biodegradation of contaminants in soil and sediments due to an inadequate supply of water to sustain proliferation, metabolism, or both (3, 6, 21). Studies of dredged sediments have also showed that PCB dechlorination ceased at an early stage after encapsulation, but the sediments still harbored dechlorinating organisms (8). To enhance and optimize PCB-dechlorinating microbial activities in dredged sediments, it is essential to understand the effects of moisture content on the dechlorination and the growth of PCB-dechlorinating microorganisms.

In this study, we investigated dechlorination of Aroclor 1248 and the population dynamics of PCB dechlorinators,

* To whom correspondence should be addressed.
(Tel) 82-55-320-3249; (Fax) 82-55-334-7092
(E-mail) envkos@ijnc.inje.ac.kr

methanogens, and sulfate-reducing bacteria (SRB) in laboratory cultures with high and low moisture contents.

Materials and Methods

Preparation of dechlorinating culture and moisture control

PCB-free, air-dried, sieved sediments from Owasco Lake, NY, were spiked with Aroclor 1248 (AccuStandard, New Haven, CT) in hexane to yield a total PCB concentration of 300 μg/g on a sediment dry-weight basis. After the hexane was evaporated, the PCB-spiked sediments were made into slurries containing 10% sediment (w/v on a dry-weight basis) with reduced minimal media (4), as previously described (17). Sediment slurries without PCBs were also made for the measurement of moisture content. Batch incubations were prepared by dispensing 50 ml of the sediment slurry into serum vials (100 ml) and sealing them with Teflon-lined rubber septa and aluminum crimp seals. All vials were autoclaved and, except for the control, inoculated with 2 ml of supernatant of sediment slurry prepared from St. Lawrence River sediments collected adjacent to a General Motors site (20).

To investigate the effect of moisture content on PCB dechlorination and population dynamics of PCB-dechlorinating microorganisms, the water layers of Aroclor 1248-spiked and non-spiked sediment vials were removed after a 4-week incubation by Pasteur pipet. The moisture was successively removed by adding and exchanging small pieces of filter paper (Whatman #2) at each sampling time. However, water layers of another set were not removed throughout the experiment. All culture vials were set up in duplicate.

Sampling and moisture content determination

At predetermined time intervals, samples were collected in an anaerobic chamber (Coy Laboratory Products). From the vials with an overlying water layer, a 2-ml portion of sediment slurry of Aroclor 1248-amended cultures was removed for PCB analysis, and 1 ml was taken and transferred into a diluent vial for MPN determination. For moisture content analysis, 2-ml samples were taken from PCB-non-amended cultures and placed in weighing dishes. Collection of these samples was done using a Pasteur pipet with a cutoff tip while the slurry was continuously mixed on a magnetic stirrer plate.

From the vials with reduced moisture content, appropriate amounts of sediments were sampled using a glass tube. Samples for PCB analysis and MPN determination were taken from vials of PCB-amended cultures, and samples for moisture content determination from non-amended cultures. Immediately after the sampling, the vials were recapped and returned for further incubation.

Moisture contents were calculated by the differences between wet weight and dry weight after oven-drying for 2 hours at 105°C.

MPN determination

Enumeration of PCB-dechlorinators, methanogens, and sulfate-reducing bacteria (SRB) in the culture vials was performed by a five-tube MPN procedure as described by Kim and Rhee (12). MPN test medium was made with the sediments spiked with 2,5,3',4'-chlorobiphenyl (CBP) (100 μg/g of dry sediment), and the diluents were made with the reduced minimal medium (4). The MPN vials were inoculated with the sediment slurry of from 10^{-1} to 10^{-9} dilution range, and incubated statically at room temperature for 12 weeks.

MPN vials showing dechlorination were counted as positive when 2,5,3',4'-CBP was dechlorinated with a concomitant increase of daughter congeners. Methane was determined by analyzing headspace samples of the MPN vials with a GC-FID (Hewlett-Packard 5890II). Vials were considered positive if methane present was two times more than in the autoclaved control. The growth of SRB was determined by the blackening of sediments. Vials with black sediments were counted as positive. The MPNs of PCB dechlorinators, methanogens, and SRB were calculated from the numbers of positive vials in consecutive dilutions by using the MPN table (11) and normalized as the MPN per gram of sediment.

PCB extraction and analysis

Sediments from the culture vials were extracted with acetone and hexane by ultrasonication as described previously (17). PCBs in the MPN test vials were extracted with acetone and hexane by shaking on an orbital shaker as described by Kim and Rhee (12). PCB analysis of extracts was performed by using a gas chromatograph (Hewlett-Packard 5890II) equipped with a ^{63}Ni electron capture detector, HP Ultra II fused silica capillary column (25 m × 0.2 mm, 0.11-μm film thickness), HP 7673 autosampler, and HP 3396 integrator. The gas chromatography conditions used have been described elsewhere (20). PCBs were quantitated on the HP Ultra II column with a calibration standard containing equal amounts of Aroclors 1221, 1016, 1254, and 1260 (0.2 μg/ml of each in hexane) as described previously (17). All of the chromatographic data were collected and processed on a microcomputer by using a HP 3365 Series II ChemStation chromatography data system. The mole percentage of PCB congeners and the average number of Cl molecules per biphenyl were calculated based on the concentration of each congener. Coeluting congeners were assumed to be present in equal proportions for the calculations.

Results

Moisture contents versus PCB dechlorination

The average moisture content of culture vials with an

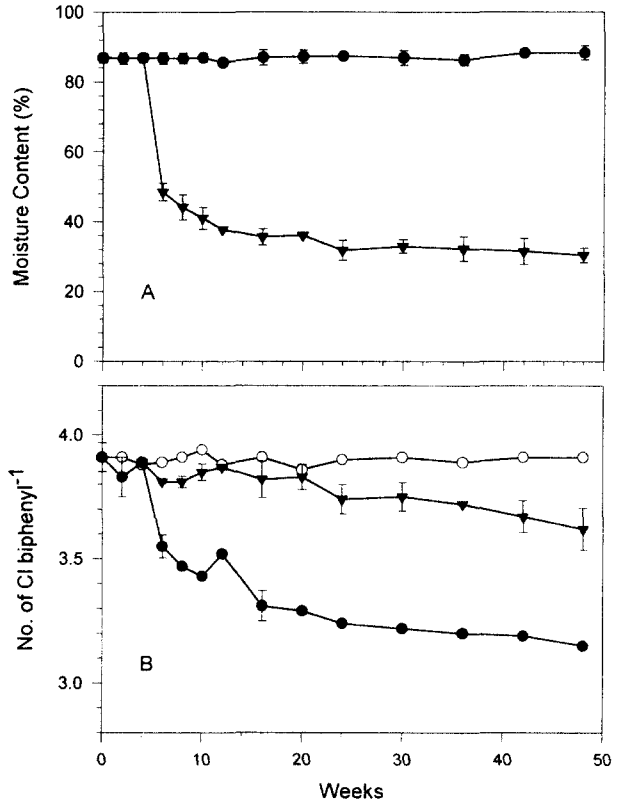

Fig. 1. Moisture content (%) (A) and Aroclor 1248 dechlorination (B), expressed as the number of Cl per biphenyl, in the sediment of an overlying water layer (●) and a reduced moisture content (▼). Each point represents the mean of duplicate vials except the autoclaved control (○).

overlying water layer was 87.1 ± 1.4% (Fig. 1a). After 4 weeks of incubation, the water layers of culture vials from another set were removed and by 6 weeks the moisture content was 48.5%. Moisture was successively removed from these vials by filter papers and the average level from 6 to 48 weeks was 36.6 ± 5.8%.

The time course of dechlorination, expressed as the total chlorines (Cls) per biphenyl, showed that the Aroclor 1248-spiked sediment slurries were dechlorinated after a 4-week lag period (Fig. 1b). After 6 weeks of incubation, 9.2% of the total Cls were removed and by 24 weeks 17.1% were removed. Analysis after 48 weeks showed that the total number of Cls per biphenyl was reduced from 3.91 to 3.15 or an overall 19.1% reduction from the original Aroclor 1248. In the sediments with reduced moisture contents, however, dechlorination occurred after a long lag period of 12 weeks (Fig. 1b). After 24 weeks, 4.3% of the total Cls were removed and by 48 weeks the total Cls was reduced only to 3.62 or an overall 7.4% reduction.

After 12 weeks of incubation, the chromatographic pattern of Aroclor 1248-spiked sediment slurry showed that decreases involved 2,4,5,4'-, 2,5,3',4'-, 2,4,3',4'-, 2,4,5,2',5'-, 2,4,5,2',4'-, 2,3,4,2',4'- + 2,3,6,2',3',6'-, and 2,3,6,2',4',5'- + 2,4,5,3',4'-CBPs with concomitant increases in 2,3-, 2,5,2'-, 2,3,2'- + 2,6,4'-, 2,5,3'-, 2,4,3'-, 2,5,4'- + 2,4,4'-, 3,4,2'-, 2,5,2',5'-, and 2,4,2',5'-CBPs (Fig. 2). By 24 weeks, there were additional decreases in congeners such as 2,3,2',5'-, 2,3,6,4'-, and 3,4,3',4'- + 2,3,6,3',4'-CBPs with an accumulation of 2,4- + 2,3-, 4,4'- + 2,4,2'-, and 2,3,6- + 2,6,3'-CBPs. After 48 weeks, 2,3,6,3'- + 2,3,2',4'- + 3,4,4'- and 2,3,4,2',3',6'- +2,3,4,3',4'-CBPs were additionally decreased. In sediments with reduced moisture contents, however, there were no congeners of which the increase or decrease of moles percent was higher than 1% compared to the control after 12 weeks of incubation (Fig. 2). By 24 weeks, only 3 congeners (2,4,5,4'-, 2,5,3',4'-, and 2,4,3',4'-CBPs) were decreased with concomitant increases in 2,3-, 2,5,3'-, and 2,5,4'- + 2,4,4'-CBPs. After 48 weeks, there were additional decreases in 2,3,4,2',4'- + 2,3,6,2',3',6'-, 3,4,3',4'- + 2,3,6,3',4'-, and 2,3,6,2',4',5'- + 2,3,6,3',4'-CBPs and increases in 2,5,2'-, 4,4'- + 2,4,2'-, 2,3,2'- + 2,6,4'-, 2,5,2',5'-, and 2,4,2',5'-CBPs. These decreased congeners, except 3,4,3',4'- + 2,3,6,3',4'-CBP, in the sediments of reduced moisture content were included within those of an overlying water layer after 12 weeks incubation.

Dechlorination of Aroclor 1248 occurred through the removal of *meta*- and *para*-Cls. In the sediment slurries, *para*-dechlorination was somewhat faster than *meta*-dechlorination in the early incubating periods, and a similar pattern of chlorine removal was seen in the sediments of reduced moisture contents throughout the incubation periods (Fig. 3). After 16 weeks, however, dechlorination occurred predominantly from the *para*-position in the sediment slurries, where *para*-Cls were reduced by 23.6% (from 0.76 to 0.58) on an average between 16 and 48 weeks and *meta*-Cls were nearly not reduced.

Population dynamics of anaerobic bacterial groups

The population sizes of PCB dechlorinators, methanogens, and SRB were estimated over time by the MPN technique (Fig. 4). 2,5,3',4'-CBP was used for the MPN test vials in this study because it was one of the most rapidly dechlorinated congeners of Aroclor 1248 (5) and dechlorinated in dechlorination-positive vials together with 2,3,4-CBP (12). Dechlorinating product of 2,5,3',4'-CBP in this study was only 2,5,3'-CBP. The population of dechlorinators in the culture of sediment slurry had a lag time of 4 weeks and increased gradually until 30 weeks, and then decreased (Fig. 4a). However, when the overlying water was removed after 4 weeks incubation, the lag period was extended to 12 weeks, and then the population increased exponentially until 30 weeks, then decreased. The periods of lag time in two conditions were similar to those of Aroclor 1248 dechlorination (Fig. 1b). Differing from the time courses of Aroclor 1248 dechlorination in cultures of slurry and reduced moisture content, however, the changes of dechlorinators in these two conditions had

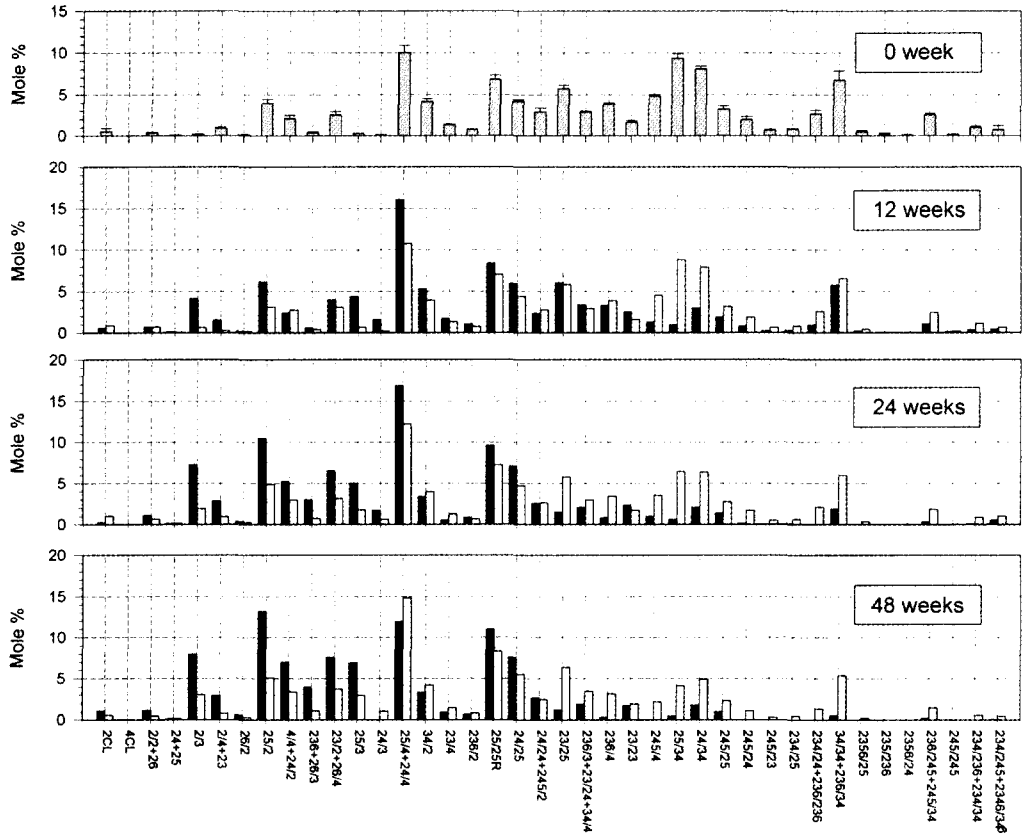

Fig. 2. Mole percents (mean ± standard deviation) of Aroclor 1248 congeners in week 0 controls (top panel) and changes of the mole percents of Aroclor 1248 congeners after 12, 24, and 48 weeks of incubation (lower 3 panels) in the sediments of an overlying water layer (■) and reduced moisture content (□).

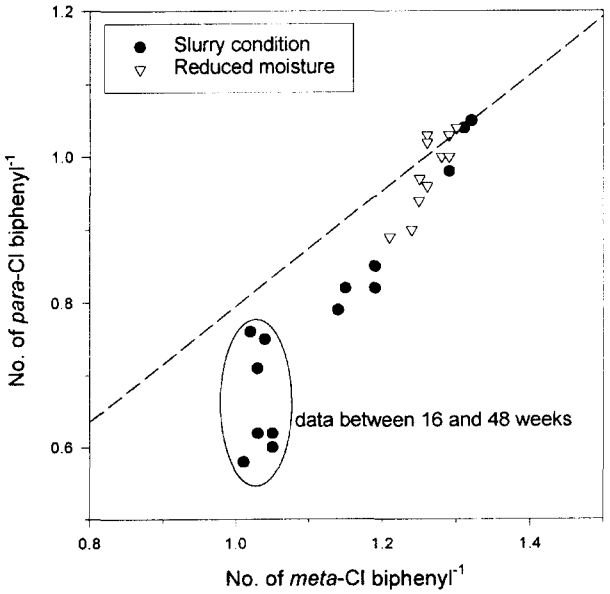

Fig. 3. Average number of *meta*-Cl versus *para*-Cl at each time point. The plot illustrates the dechlorination process beginning at the upper right and progressing toward the lower left. The dashed line represents a proportional removal of *meta*- and *para*-Cls from Aroclor 1248.

similar patterns over time. Since Aroclor 1248 was actively dechlorinated between 4 and 24 weeks in the sediment slurries (Fig. 1), the growth rate of dechlorinators was calculated for this period. The population of dechlorinators increased by 3 orders of magnitude, from 1.4×10^2 to 2.2×10^5 cells/g of sediment or a growth rate of 1.6×10^3 cells/g of sediment/day. For this exponential phase of dechlorination, the specific growth rate and the doubling time of dechlorinators were 11.4 days^{-1} and 0.06 days, respectively. The rate of dechlorination was 4.9×10^{-9} mol of Cl/g of sediment/day, based on the changes in the average number of Cl molecules per biphenyl. The growth yield of dechlorinators was calculated as 3.3×10^{11} cells/mol of Cl dechlorinated. Although the population of dechlorinators reached the maximum at 30 weeks in the sediments of reduced moisture (Fig. 4a), Aroclor 1248 was gradually dechlorinated until 48 weeks of incubation (Fig. 1b). Due to this discrepancy in low moisture conditions, we calculated the growth rate of dechlorinators by the population changes between the end of the lag period of dechlorination (12 weeks) and the time for maximum population of dechlorinators (30 weeks). During this period, dechlorinators also increased by about 3 orders of magnitude, from 5.0×10^3 to $6.5 \times$

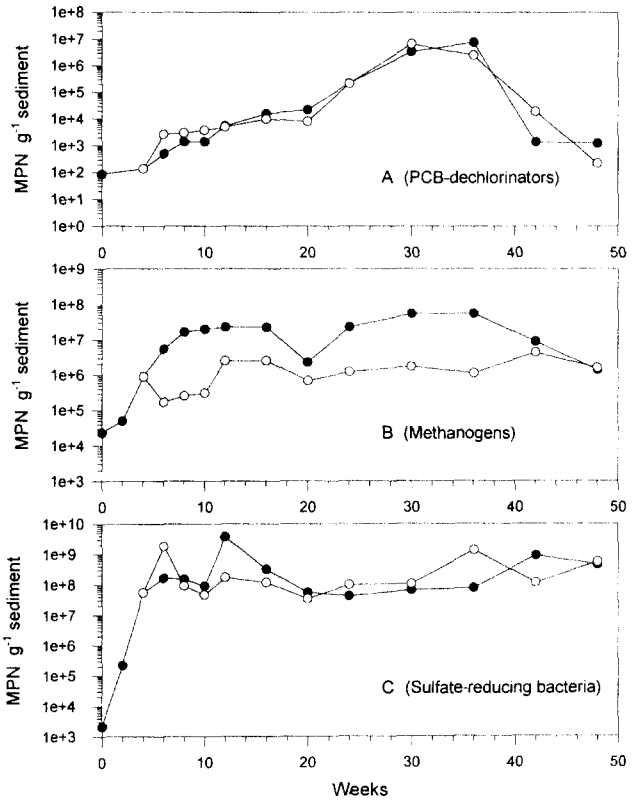

Fig. 4. Growth dynamics of PCB-dechlorinators (A), methanogens (B), and sulfate-reducing bacteria (C) in sediment cultures with an overlying water layer (●) and reduced moisture content (○).

10^6 cells/g of sediment and the growth rate was 5.2×10^4 cells/g of sediment/day. The specific growth rate and the doubling time of dechlorinators were the same as those in the sediment slurry, 10.4 $days^{-1}$ and 0.07 days, respectively. The dechlorination rate and the growth yield were calculated as 8.4×10^{-10} mol of Cl/g of sediment/day and 6.2×10^{13} cells/mol of Cl dechlorinated, respectively.

The growth of methanogens in cultures of sediment slurry and reduced moisture condition had a lag time of 2 and 10 weeks, respectively, which was shorter than that of dechlorinators (Fig. 4b). After the lag periods, the number of methanogens in sediment slurry increased from an initial level of 2.3×10^4 to 1.6×10^7 cells/g of sediment at 8 weeks, which was about 4 orders of magnitude higher than that of dechlorinators. After the exponential growth phase, the size of methanogens was maintained up to 36 weeks, then decreased. The population of methanogens in low moisture, however, did not show a distinct exponential growth phase. After 12 weeks incubation, it increased only to 2.5×10^6 cells/g of sediment, which was about 1 order of magnitude lower than that of sediment slurry, and maintained its maximum level up to 48 weeks. After 48 weeks incubation, the populations of methanogens in these two conditions were at a similar level, which was still about 3 orders of magnitude higher than those of dechlorinators.

SRB grew rapidly during the first 4 weeks, reaching their highest level earlier than dechlorinators and methanogens (Fig. 4c). The number of SRB increased from an initial level of 2.1×10^3 to 5.5×10^7 cells/g of sediment after 4 weeks; at its maximum, the population size was about 2 to 3 orders of magnitude higher than those of dechlorinators and methanogens. However, unlike dechlorinators and methanogens, the size of the SRB population changed little after reaching the upper plateau, maintaining its maximum level up to 48 weeks. The numbers of SRB in the two conditions showed little difference.

Discussion

Sediment microorganisms used in this study were able to reductively dechlorinate Aroclor 1248 to a mixture composed largely of tri-, tetra-, and dichlorinated congeners having 52.5, 29.0, and 15.7 mole %, respectively, in the sediment slurry of an overlying water layer after 48 weeks. During the early incubation periods, *para*- and *meta*-Cl were proportionally removed; however, *para*-Cl was preferentially dechlorinated at the late periods and *meta*-rich congeners such as 2,3-, 2,5,2'-, 4,4'- + 2,4,2'-, 2,3,2'- + 2,6,4'-, 2,5,4'- + 2,4,4'-, 2,5,2',5'-, and 2,4,2',5'-CBPs accumulated. The dechlorination rate and extent of Aroclor 1248 in this study were similar to those of Sokol *et al.* (20), but lower than Kim and Rhee's (12) by using the sediments taken from the same general areas of the GM site in the St. Lawrence River. In the study of Kim and Rhee (12), after only 6 weeks of incubation the average Cl per biphenyl was reduced to 2.8 and the major dechlorination peaks were di- and trichlorinated congeners. Since the experimental protocols of these studies were the same, it was supposed that the discrepancy might be caused by the differences of the sediment inoculum: composition or population size of microorganisms. Comparing the initial population size of the dechlorinator, it was smaller in this study (1.0×10^2 cells/g of sediment) than that of Kim and Rhee (12) (2.5×10^5 cells/g of sediment), and the exponential periods (20 weeks) were much longer. Despite similar levels of specific growth rate and dechlorination rate, the growth yield (3.3×10^{11} cells/mole of Cl dechlorinated) of Aroclor 1248 dechlorination in this study was about 2 orders of magnitude lower than that of Kim and Rhee (12) (4.2×10^{13} cells/mole of Cl dechlorinated). Thus, among the conventional explanations of the lag period (3), building a sufficient population size from a small initial population of dechlorinators may be the main reason for the slower rate and the lesser extent of dechlorination of Aroclor 1248 in the present study.

Water can act as one of the limiting factors for the reductive dechlorination of PCBs in sediments of low

moisture content, since the source of hydrogen atoms for the reductive dechlorination of PCBs is the proton from water (16). Degradation of hydrophobic organic compounds (HOCs) such as PCBs is often limited by their availability, but little is known about the effect of moisture on the bioavailability of HOCs in sediments. In soil, rates of carbofuran biodegradation also decreased as a function of decreasing soil moisture, primarily due to the inhibitory effects of dessication on carbofuran-degrading microorganisms, but decreasing soil moisture had little or no effect on carbofuran bioavailability (19). In this study, the reduction of moisture content decreased the rate and extent of Aroclor 1248 dechlorination but did not inhibit the growth of PCB dechlorinators.

In spite of similar specific growth rates, the dechlorination rate of sediment slurry with an overlying water layer was about 5 times faster than that of low-moisture sediments, as expected, but the growth yield of the slurry is about 2 orders of magnitude lower. How could PCB dechlorinators maintain a similar population size in the reduced moisture contents? Dechlorinating microorganisms can derive energy in anaerobic respiration, as indicated by reductive dechlorination of 3-chlorobenzoate (9, 10), and the growth of PCB dechlorinators requires the presence of PCBs (12). Addition of some carbon and energy sources has enhanced PCBs dechlorination (1, 2, 14, 15). Thus, it can be thought that dechlorinators may have a preference for energy sources other than PCBs in sediments with low moisture levels. The sediments used in this study have an organic content of 90 mg/g (17). Dechlorinators in low moisture sediments may proliferate with the consumption of other organic sources as much as they do in slurry, or dechlorinators can tolerate the low moisture and grow in MPN test vials which have sediment slurry.

MPN of methanogens was affected by the reduction of moisture content and the population in low moisture conditions was 1 to 2 orders of magnitude lower than in the slurry condition. The changes of average Cl number per biphenyl over time showed a closer relationship with the variation of methanogenic population ($r = -0.65$, $p<0.01$) than those of dechlorinators and SRB ($p>0.30$). Some investigators have been using 2-bromoethane sulfonate (BES), an inhibitor of methanogenesis, and found that the addition of BES reduced the dechlorination rate and extent of PCBs (14, 22, 23). On the other hand, amendment of BES had little effect on 2,3,4-CBP dechlorination (18). These conflicting results of BES treatment indicate the presence of different microorganisms for the *meta* dechlorination of 2,3,4-CBP (18). Thus, like the suggestion of Ye *et al.* (23), the results of this study also show that methanogens are among the physiological groups capable of PCB dechlorination.

The results indicate that the rate and extent of PCB dechlorination are in part determined by moisture content of sediments, probably due to the preference of energy source in sediments other than PCBs. Therefore, although the underlying mechanism for the preference remains unclear, optimization of moisture content in dredged sediments is required for the bioremediation of PCBs.

Acknowledgment

This work was supported by a 1999 Inje University research grant.

References

1. Abramowicz, D.A., M.J. Brennan, H.M. Van Dort, and E.L. Gallagher. 1993. Factors influencing the rate of polychlorinated biphenyl dechlorination in Hudson River sediments. *Environ. Sci. Technol.* 27, 1125-1131.
2. Alder, A.C., M.M. Häggblom, S.R. Oppenheimer, and L.Y. Young. 1993. Reductive dechlorination of polychlorinated in anaerobic sediments. *Environ. Sci. Technol.* 27, 530-538.
3. Alexander, M. 1994. Biodegradation and bioremediation. p. 196-225. Academic Press, San Diego, CA.
4. Balch, W.E., G.E. Fox, L.J. Magrum, C.R. Woese, and R.S. Wolfe. 1979. Methanogens: reevaluation of a unique biological group. *Microbiol. Rev.* 43, 260-296.
5. Bedard, D.L. and J.F. Quensen III. 1995. Microbial reductive dechlorination of polychlorinated biphenyls, p. 127-216. *In* L.Y. Young and C.E. Cerniglia (eds), Microbial transformation and degradation of toxic organic chemicals, Wiley-Liss, New York, N.Y.
6. Boul, H.L. 1996. Effect of soil moisture on the fate of radiolabelled DDT and DDE *in vitro*. *Chemosphere* 32, 855-866.
7. Chang, B.-V., L.-N. Yeh, and S.-Y. Yuan. 1996. Effect of a dichlorophenol-adapted consortium on the dechlorination of 2,4,6-trichlorophenol and pentachlorophenol in soil. *Chemosphere* 33, 303-301.
8. Cho, Y.-C., O.-S. Kwon, R.C. Sokol, C.M. Bethoney, and G.-Y. Rhee. 2001. Microbial PCB dechlorination in dredged sediments and the effect of moisture. *Chemosphere* 43, 1119-1126.
9. Dolfing, J. 1990. Reductive dechlorination of 3-chlorobenzoate is coupled to ATP production and growth in an anaerobic bacterium, strain DCB-1. *Arch. Microbiol.* 153, 264-266.
10. Dolfing, J. and J.M. Tiedje. 1987. Growth yield increase linked to reductive dechlorination in a defined 3-chlorobenzoate degrading methanogenic coculture. *Arch. Microbiol.* 149, 102-105.
11. Greenberg, A.E., L.S. Clesceri, and A.D. Eaton. 1992. Standard Methods for Examination of Water and Wastewater, 18th ed. APHA, AWWA, WEF, Washington D. C.
12. Kim, J. and G.-Y. Rhee. 1997. Population dynamics of polychlorinated biphenyl-dechlorinating microorganisms in contaminated sediments. *Appl. Environ. Microbiol.* 63, 1771-1776.
13. Mohn, W.W. and J.A. Tiedje. 1992. Microbial reductive dehalogenation. *Microbiol. Rev.* 56, 482-507.
14. Morris, P.J., W.W. Mohn, J.F. Quensen III, J.M. Tiedje, and S.A. Boyd. 1992. Establishment of a polychlorinated biphenyl-degrading enrichment cultures with predominantly *meta* dechlorination. *Appl. Environ. Microbiol.* 58, 3088-3094.
15. Nies, L. and T.M. Vogel. 1990. Effects of organic substrates on

dechlorination of Aroclor 1242 in anaerobic sediments. *Appl. Environ. Microbiol.* 56, 2612-2617.

16. Nies, L. and T.M. Vogel. 1991. Identification of the proton source for the microbial reductive dechlorination of 2,3,4,5,6-pentachlorobiphenyl. *Appl. Environ. Microbiol.* 57, 2771-2774.
17. Rhee, G.-Y., B. Bush, C.M. Bethoney, A. DeNucci, H.-M. Oh, and R.C. Sokol. 1993. Reductive dechlorination of Aroclor 1242 in anaerobic sediments: pattern, rate, and concentration dependence. *Environ. Toxicol. Chem.* 12, 1025-1032.
18. Rhee, G.-Y., B. Bush, C.M. Bethoney, A. DeNucci, H.-M. Oh, and R.C. Sokol. 1993. Anaerobic dechlorination of Aroclor 1242 as affected by some environmental conditions. *Environ. Toxicol. Chem.* 12, 1033-1036.
19. Shelton, D.R. and T.B. Parkin. 1991. Effect of moisture on sorption and biodegradation of carbofuran in soil. *J. Agr. Food Chem.* 39, 2063-2068.
20. Sokol, R.C., O.-S. Kwon, C.M. Bethoney, and G.-Y. Rhee. 1994. Reductive dechlorination of polychlorinated biphenyls in St. Lawrence River sediments and variations in dechlorination characteristics. *Environ. Sci. Technol.* 28, 2054-2064.
21. Wardell, L.J. 1995. Potential for bioremediation of fuel-contaminated soil in Antarctica. *J. Soil Contam.* 4, 111-121.
22. Williams, W.A. 1994. Microbial reductive dechlorination of polychlorinated biphenyls in heat treated and bromoethane sulfonate treated anaerobic slurries. *Chemosphere* 28, 2269-2284.
23. Ye, D., J.F. Quensen III, J.M. Tiedje, and S.A. Boyd. 1992. Anaerobic dechlorination of polychlorobiphenyls (Aroclor 1242) by pasteurized and ethanol treated microorganisms from sediments. *Appl. Environ. Microbiol.* 58, 1110-1114.